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Cartilage-Derived Retinoic Acid–Sensitive Protein and Type II Collagen Expression During Fracture Healing Are Potential Targets for Sox9 Regulation

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ABSTRACT

Cartilage-derived retinoic acid-sensitive protein (CD-RAP) and mRNA were examined in the mouse fracture model by immunohistochemistry and Northern blot analysis and compared with the expression of type II collagen. We also studied the expression of the transcription factor Sox9, reported to enhance type II collagen and CD-RAP gene expression in vitro. CD-RAP was first detected in immature chondrocytes on day 5. Intense signals for CD-RAP were found in fracture cartilage on days 7 and 9. CD-RAP decreased at the phase of endochondral ossification. Throughout fracture healing, CD-RAP was detected in cartilage and not in bone or fibrous tissue, thus CD-RAP may be a molecular marker of cartilage formation during fracture healing. Northern blot analysis revealed similar changes in CD-RAP and type II collagen mRNA levels. However, with respect to protein levels, CD-RAP decreased faster than type II collagen implying the stability is lower than type II collagen. Increased levels of Sox9 mRNA and protein were detected on day 5 and coincided with the initial increase of CD-RAP and type II collagen mRNAs. Sox9 mRNA levels declined with the progress of chondrocyte hypertrophy, followed by a concomitant decrease in CD-RAP and type II collagen mRNA levels. These changes in Sox9 expression compared with the cartilage-specific genes (CD-RAP and type II collagen) suggest that cell differentiation during fracture healing may be controlled by specific transcriptional factors which regulate phenotypic changes of the cells. (J Bone Miner Res 1999;14:1891–1901)

INTRODUCTION

FRACTURE HEALING is a sequential event following bone injury and is believed to recapitulate embryogenic chondrogenesis and osteogenesis. The process of fracture healing is recognized as a sequence of three phases: inflammation (hematoma), callus formation (repair), and remodeling.[1,2] Considering these events from the view of cell differentiation, fracture repair progresses through two different pathways. One is intramembranous ossification and the other is endochondral ossification.[3,4] In this decade, the progress of molecular biology and its application to animal fracture models have dramatically changed and enhanced the knowledge of the repair mechanism after bone fracture. The expression of collagens type I, II, III, V, VI, IX, X, and XI have been reported during cell differentiation at the fracture site.[5–15] The alternatively spliced forms of α1 type II collagen, type IIA, and type IIB, were shown to be expressed during the fracture repair process, reflecting the normal developmental sequence.[14] The expression of noncollagenous extracellular matrix proteins including osteonectin, osteopontin, osteocalcin, matrix Gla protein, alkaline phosphatase, and aggrecan were shown to change during cartilage and bone differentiation as a result of fracture healing.[9–11,13,16] These findings have suggested that changes in cell phenotypes and in the structure of the extracellular matrix are due to the regulation of genes at the fracture site.

Cartilage-derived retinoic acid–sensitive protein (CD-RAP) is a secreted protein consisting of 130 amino acids in bovine, rat, and mouse and 131 amino acids in human. As a result of specific regulation by retinoic acid, bovine CD-

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RAP mRNA was identified and subsequently isolated from cultured chondrocytes using differential display PCR.[17] Melanoma inhibitory activity (MIA) was independently cloned from a human melanoma cell line, and is the human homolog of CD-RAP.[18] The observation of CD-RAP expression in the mouse embryo demonstrates its specific distribution in chondrocytes.[17,19] The expression of CD-RAP/MIA was also reported in human melanoma, rat, and human chondrosarcoma and in rat and human mammary carcinoma.[18–22] These findings suggest that CD-RAP may have an important role in chondrogenesis and carcinogenesis. Information on the function and regulation of this new protein is lacking since the sequence of CD-RAP gene encodes no recognized protein motifs except the secretory signal sequence.

Recently, it was shown that the transcription factor Sox9, a close relative of the sex-determining region Y protein (SRY), has an activating role in chondrogenesis through the enhancement of type II collagen gene expression.[23–26] Mutations in the Sox9 gene cause campomelic dysplasia with severe abnormalities in skeletal structures.[27,28] Our in vitro experiments revealed that Sox9 regulates CD-RAP by binding to the consensus region in the CD-RAP promoter.[29] These findings lead us to the hypothesis that Sox9 may be involved in chondrogenesis during fracture healing by increasing the expression of both type II collagen and CD-RAP genes.

To test this hypothesis, we investigated the expression of CD-RAP, type II collagen, and Sox9 mRNAs by Northern blot analysis, and the tissue distribution of CD-RAP, type II collagen and Sox9 by immunohistochemistry in the mouse fracture model.

MATERIALS AND METHODS

Fracture model

Sixty-five C57BL/6 J, 8- to 9-week-old, male mice were used in the present study. C57BL/6 J mice were purchased from the Jackson Laboratory (Bar Harbor, ME, U.S.A.). A closed fracture of the left tibia was produced in each mouse under anesthesia using the rat femur fracture device described by Bonnarens and Einhorn,[30] except that it was modified for use in the mouse tibia. An intraperitoneal injection (75 mg/kg body weight) of pentobarbital sodium was given for anesthesia. Skin incision was made on the proximal tibia and a 27 G needle was introduced into the intramedullary canal of the tibia. The prenailed lower leg bone was fractured by an impact device. Mice were killed 0, 2, 3, 5, 7, 9, 14, and 21 days after surgery by cervical dislocation under anesthesia. For immunohistochemistry and Northern blot analysis, three to five left tibiae were used on each day point. Tissue sections were also examined by staining with hematoxylin and eosin and with toluidine blue as routine histology. This study protocol was reviewed and approved by the Animal Studies Committee of Washington University.

Immunohistological analysis

Each lower leg was fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 16 h and decalcified by Formical-2000 (Decal Chemical Corporation, Congers, NY, U.S.A.) for 24 h. After dehydration, the lower leg samples were embedded in paraffin, sectioned to 5 μm and mounted on Superfrost/Plus Microscope slides (Fisher Scientific, Pittsburgh, PA, U.S.A.). Immunochemical staining was performed by the three-step avidin-biotin complex method using the Dako LSAB Kit (Dako Corp., Carpinteria, CA, U.S.A.). After rehydration, the slides were incubated in 3% hydrogen peroxide for 15 minutes at room temperature. Sections were digested with 1% hyaluronidase for 45 minutes at 37°C. The primary antibody was applied to the sections and incubated for 45 minutes at room temperature. Antibodies against human CD-RAP/MIA,[19] bovine type II collagen[31] and human Sox9[23] were used at a dilution of 1:500, 1:100, and 1:30, respectively. After addition of secondary antibody for 30 minutes at room temperature, the slides were incubated in streptoavidin solution for 30 minutes at room temperature. The antigen site was visualized by 3-amino-9-ethylcarbazole as a chromogen resulting a reddish brown precipitation. To identify proliferating chondrocytes at the fracture site, the proliferating cell nuclear antigen (PCNA) was detected by Zymed PCNA Staining kit (Zymed Laboratories, Inc., San Francisco, CA, U.S.A.). The antibody recognizing human CD-RAP/MIA was generated by Boehringer-Mannheim (Penzburg, Germany). The bovine type II collagen antibody was provided by Dr. Michael Cremer and the human Sox9 antibody was provided by Dr. Veronique Lefebvre and Dr. Benoit de Crombrugghe.

Northern blot analysis

To remove growth cartilage and articular cartilage, the proximal and distal 1/4 of the lower leg was discarded during the dissection and the middle 2/4 was used for RNA extraction. Each sample was pulverized in liquid nitrogen. Total RNA was extracted using TRIZOL Reagent (Life Technologies, Grand Island, NY, U.S.A.) following the manufacturer's instructions.

Total RNA (15 μg) was electrophoresed in a 1% agarose gel containing 6% formaldehyde and 1× MOPS buffer (1× MOPS buffer = 20 mM MOPS, 5 mM sodium acetate, and 1 mM EDTA) and transferred to Hybond N+ nylon membrane (Amersham, Arlington Heights, IL, U.S.A.) by capillary blotting with 20× SSC (1× SSC = 0.15 M NaCl and 15 mM sodium citrate). The RNA was cross-linked to the membrane by a CL-1000 Ultraviolet Crosslinker (UVP, Upland, CA, U.S.A.). The membrane was prehybridized at 42°C for 2 h in a solution containing 50% formamide, 5× SSPE (1× SSPE = 0.15 M NaCl, 10 mM sodium phosphate ans 1 mM EDTA), 5× Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured herring sperm DNA. Hybridization was performed at 42°C for 20 h with the labeled probe in the same solution used for prehybridization. After hybridization, the membrane was washed twice at room temperature for 10 minutes in 2× SSPE and 0.1% SDS and twice at 65°C for 10 minutes in 2× SSPE and 0.1% SDS. The membrane was exposed to Hyperfilm MP (Amersham, Arling-

ton Heights, IL, U.S.A.). The density of each band on the autoradiogram was estimated by an image analyzer ISS SepraScan 2001 (Integrated Separation Systems, Natick, MA, U.S.A.)

The cDNAs for mouse CD-RAP,[17] mouse type II collagen,[32] mouse Sox9[23] and mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were labeled with [^{32}P]dCTP using a random primed labeling kit (Life Technologies, Grand Island, NY, U.S.A.). Mouse type II collagen cDNA was provided by Dr. Eero Vuorio. CD-RAP cDNA was labeled using the specific primers, 5'-CGCGGATCCGAGCTCACTGGCAGTAGAAATCC-3' and 5'-CGCGAATTCATGCCCAAGCTGGCTGAC-CGGAA-3' instead of random primers. The probe for GAPDH was used to normalize the amount of mRNA in each sample lane.

RESULTS

Histologic observations of fracture healing

The mouse fracture model showed the sequential differentiation of cells that are involved in cartilage and bone formation at the fracture site (Figs. 1 and 2). This method of fracture produces very little soft tissue damage and leads to a reproducible, organized healing process. As early events, the migration of inflammatory cells, the thickening of periosteum, and the formation of hematoma were observed at days 2 and 3 after fracture (Figs. 1A and 1B). On day 5, immature chondrocytes were identified at the fracture site and toluidine blue staining showed metachromasia which indicates the production of cartilage matrix (Figs. 1C and 2A). Also on day 5, new trabecular bone, which was formed by intramembranous ossification, was first observed along cortical bone near the fracture site. On days 7 and 9, many mature chondrocytes were observed (Figs. 1D and 1E) and the extracellular matrix produced by these chondrocytes showed intense metachromasia (Figs. 2B and 2C). The chondrocytes on day 9 were more differentiated toward hypertrophy than those of day 7. The area of new trabecular bone was also increased on days 7 and 9. On day 14, a bone bridge was formed between distal and proximal cortical bone (Fig. 1F). The area of cartilage decreased as endochondral ossification progressed and a few hypertrophic chondrocytes were identified in the fracture callus (Fig. 2D). On day 21, bone union was complete and low levels of cartilage matrix were observed at the fracture site (Figs. 1G and 2E).

Immunohistological analysis

At the fracture site, no type II collagen (Figs. 3A and 3B) or CD-RAP (Figs. 4A and 4B) was detected on day 2 or 3. On day 5, type II collagen was first observed in immature chondrocytes (Fig. 3C), as was CD-RAP but at lower levels than type II collagen (Fig. 4C). On days 7 and 9, high levels of CD-RAP (Figs. 4D and 4E) and type II collagen (Figs. 3D and 3E) were identified in mature and hypertrophic chondrocytes. The area in which these two molecules were expressed was consistent with the metachromatic cartilage matrix stained with toluidine blue. On day 14, the signal area and intensity for CD-RAP decreased with the progress of hypertrophy and endochondral ossification (Fig. 4F). In contrast, although the area of type II collagen decreased with the progression of endochondral ossification, levels in the hypertrophic chondrocytes appeared to remain the same. Compared with CD-RAP, more type II collagen was detected at the endochondral ossification site (Fig. 3F). On day 21, the signal for CD-RAP disappeared from the fracture site (Fig. 4G). However, some type II collagen was still detected in the remaining cartilage matrix (Fig. 3G). Immunohistochemical detection of type II collagen provides a molecular marker distinguishing between bone formed by intramembranous and endochondral ossification. The tissue section from the day 14 specimen (Fig. 3F) clearly shows remnants of type II collagen in the extracellular matrix of endochondral bone, but they are not present in the adjacent bone formed from callus in the periosteum.

During fracture repair, transcription factor Sox9 was not detected on day 2 (data not shown) or day 3 (Fig. 5E) by immunohistochemistry. PCNA-positive cells were found around the fracture site and in the muscles on days 2 (data not shown) and 3 (Fig. 5A). On day 5, many PCNA-positive cells were seen in immature chondrocytes (Fig. 5B). On the same day, marked signals of Sox9 were observed in immature chondrocytes (Figs. 5F and 5I). On days 7 and 9, Sox9 was still detected in mature and hypertrophic chondrocytes (Figs. 5G, 5H, 5J, and 5K). Sox9 levels decreased with the progress of hypertrophy, which is concomitant with the disappearance of PCNA signals (Figs. 5C, 5D, 5G, 5H, 5J, and 5K). Sox9 was barely detectable in the hypertrophic chondrocytes on day 14 (data not shown). During fracture healing, Sox9 was localized only in the nuclei of chondrocytes (Figs. 5I–5K). Neither osteoblastic cells nor fibroblastic cells showed a signal for Sox9 throughout the fracture healing process.

Northern blot analysis

Sequential changes in the expression of type II collagen, CD-RAP, and Sox9 mRNAs were detected by Northern blot analysis (Fig. 6). Figure 7 shows changes in the amount of each mRNA relative to levels of GAPDH mRNA. A low level of Sox9 mRNA was detected on days 2 and 3, whereas the expression of CD-RAP or type II collagen mRNA was not detected at this time. On day 5, a dramatic increase in the expression of Sox9 mRNA was noted with the appearance of immature chondrocytes. In addition, low level expression of CD-RAP and type II collagen mRNAs was first detected on day 5. On day 7, the expression of Sox9, CD-RAP, and type II collagen showed peak levels, with the increase of mature chondrocytes at the fracture site. On day 9, the expression of Sox9 mRNA began to decrease, but the expression of CD-RAP and type II collagen mRNA remained at high levels. On day 14, with the progress of hypertrophy and endochondral bone formation, the expression of CD-RAP and type II collagen mRNAs began to decline. On day 21, the expression of Sox9, CD-RAP, and type II collagen mRNAs had all decreased.

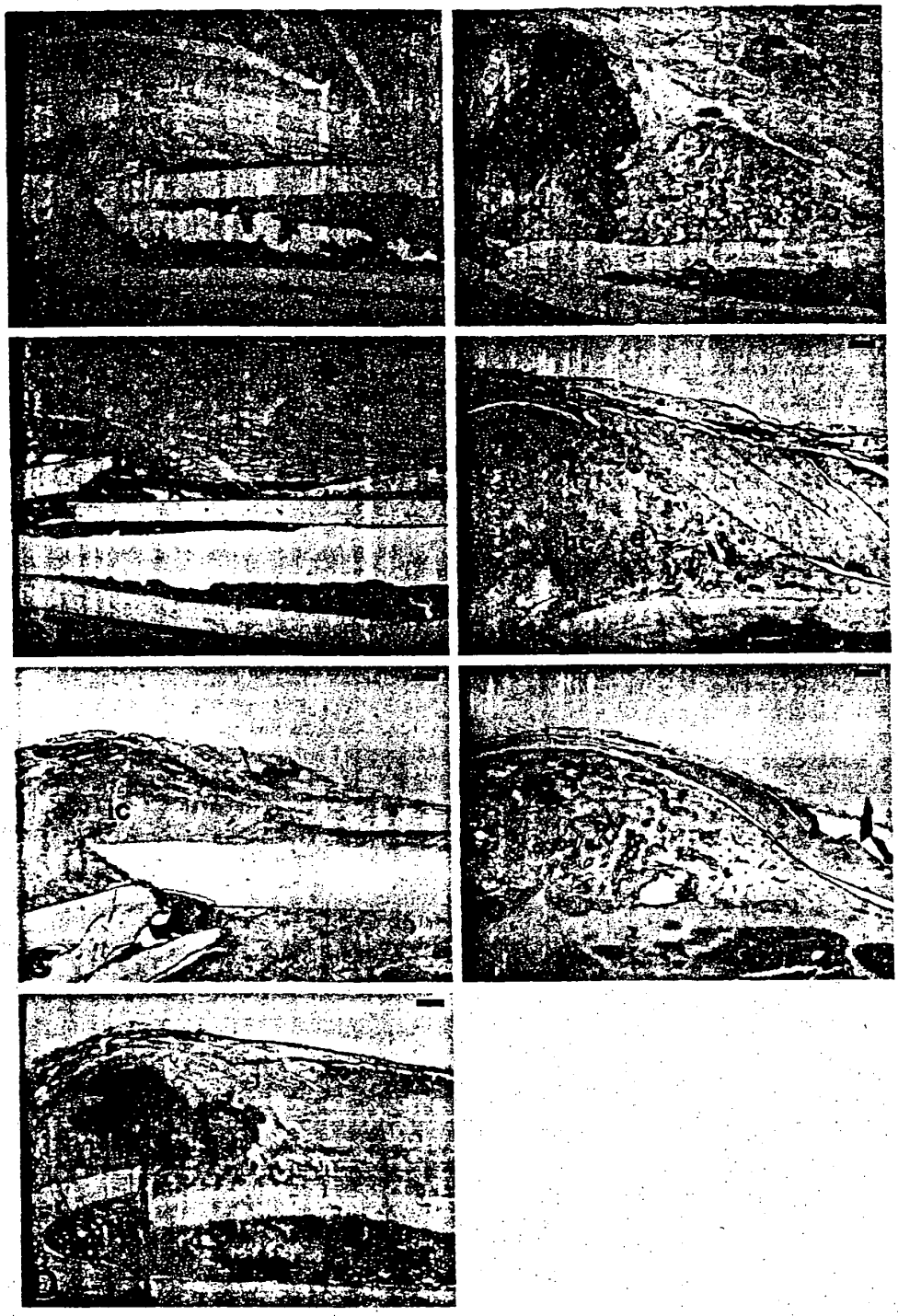

FIG. 1. Photomicrographs showing the histologic features of the mouse tibial fracture healing on days 2 (A), 3 (B), 5 (C), 7 (D), 9 (E), 14 (F), and 21 (G). Sections were stained with hematoxylin and eosin. Specific regions and cells were indicated as follows: muscle (ms), medullary canal (mc), cortical bone (b), periosteum (p), hematoma (h), inflammatory cells (if), immature chondrocytes (ic), mature chondrocytes (mc), hypertrophic chondrocytes (hc), and new trabecular bone (t). Bar, 200 μm.

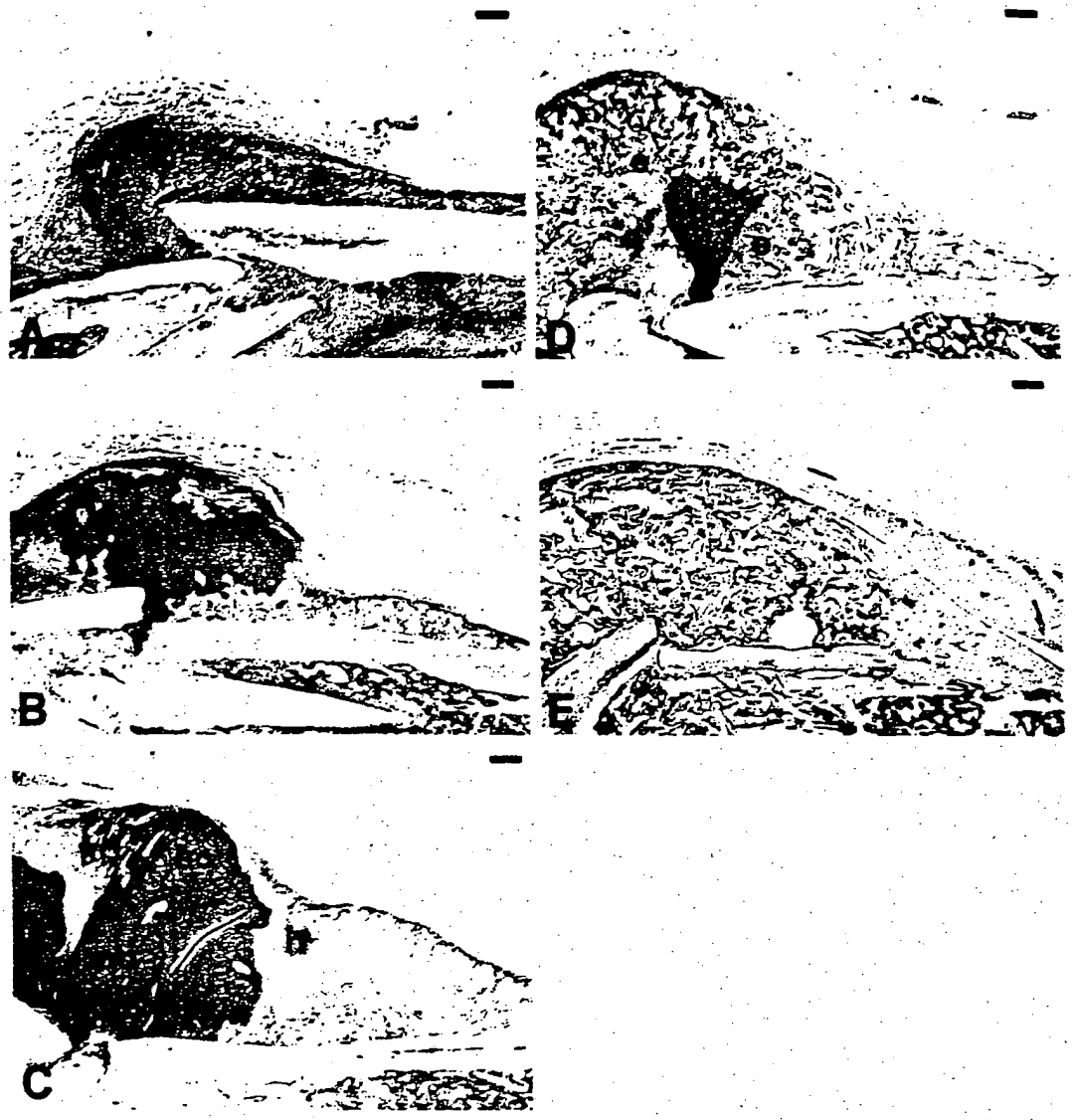

FIG. 2. Photomicrographs showing the cartilage matrix in the fracture callus. Sections were stained with toluidine blue. (A) Metachromasia was first observed in immature chondrocytes (ic) on day 5. (B) Strong metachromasia was identified on day 7 with the appearance of mature chondrocytes (mc). (C) Hypertrophic chondrocytes (hc) increased in the fracture cartilage on day 9. (D) Cartilage decreased with the progress of endochondral ossification (e) on day 14. (E) A little cartilage matrix was observed in the fracture callus on day 21. Bar, 200 μm.

DISCUSSION

We have used a rodded mouse tibia fracture as a model for fracture repair showing the histologic progression of healing and also the temporal and spatial expression of a new matrix molecule, CD-RAP, and the DNA transcription factor, Sox9. This fracture model showed a similar healing process to the rat fracture model[9,30] and the mouse model described by Hiltunen et al.[33] CD-RAP is a novel protein isolated from cultured chondrocytes as a gene regulated by retinoic acid.[17] The sequence of this gene encodes no recognized protein motifs except the secretory signal sequence. In this study, we traced the sequential changes in the expression of CD-RAP during fracture healing by immunohistochemistry and Northern blot analysis. The tissue distribution of CD-RAP was strictly limited to the cartilage at the fracture site and no staining was observed in other tissues such as bone and fibrous structures. These results are consistent with the previous findings that showed specific expression of CD-RAP in the embryonic cartilage.[17,19] These observations indicate that CD-RAP is re-expressed during fracture healing to recapitulate embryogenic differentiation leading to the structural and functional repair of damaged bone. These phenomena are also documented in other cartilage-specific molecules like type II, IX, X, and XI collagen and aggrecan.[5–15] Thus, CD-RAP may be a new marker of cartilage formation during fracture healing.

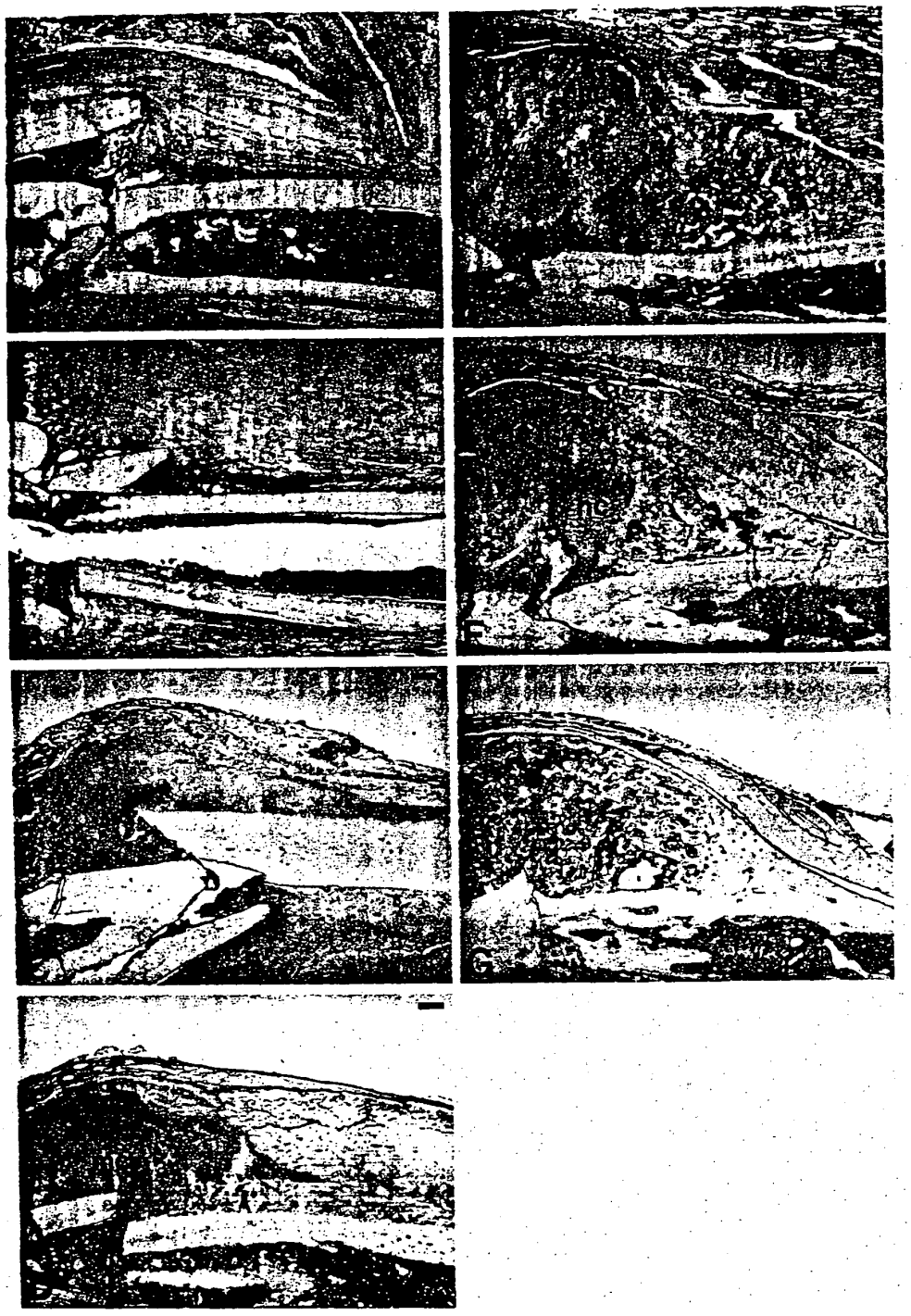

FIG. 3. Immunostaining for type II collagen on fracture callus on days 2 (A), 3 (B), 5 (C), 7 (D), 9 (E), 14 (F), and 21 (G). Specific regions and cells were indicated as follows: immature chondrocytes (ic), mature chondrocytes (mc), hypertrophic chondrocytes (hc), endochondral ossification (e). Type II collagen was first observed in immature chondrocytes on day 5. Intense signals were identified on days 7 and 9 in the fracture cartilage. The area of type II collagen signals decreased with the progression of endochondral ossification on day 14. Low levels of type II collagen remain at the site of endochondral ossification on days 14 and 21. Bar, 200 μm.

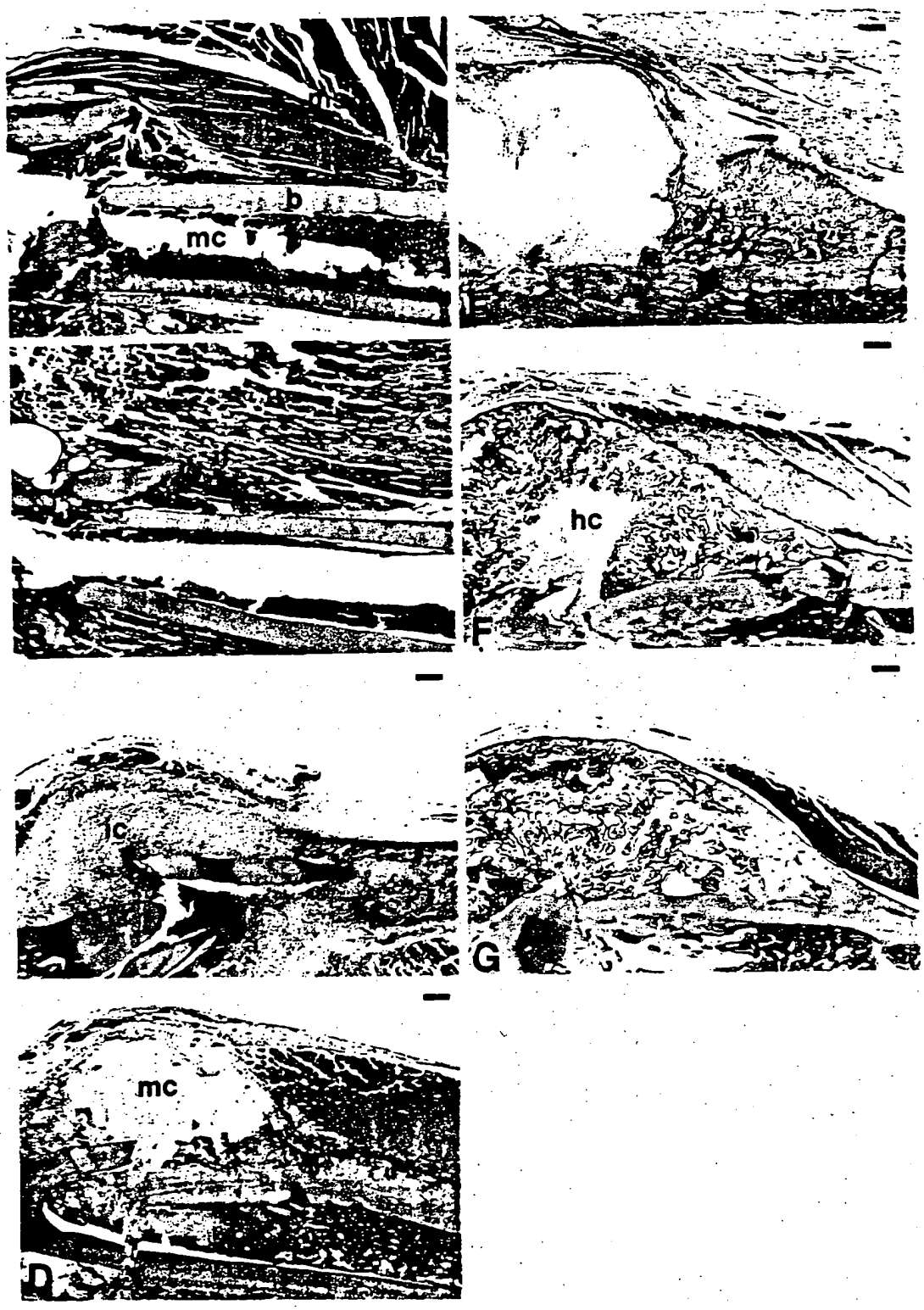

FIG. 4. Immunostaining for CD-RAP on fracture callus on days 2 (A), 3 (B), 5 (C), 7 (D), 9 (E), 14 (F), and 21 (G). Specific regions and cells were indicated as follows: immature chondrocytes (ic), mature chondrocytes (mc), hypertrophic chondrocytes (hc), endochondral ossification (e). Faint signals of CD-RAP were first observed in immature chondrocytes on day 5. Intense signals of CD-RAP were detected on day 7 and 9 in the fracture cartilage. The signals decreased with the progress of hypertrophy and endochondral ossification on day 14. CD-RAP was not detected on day 21. Bar, 200 μm.

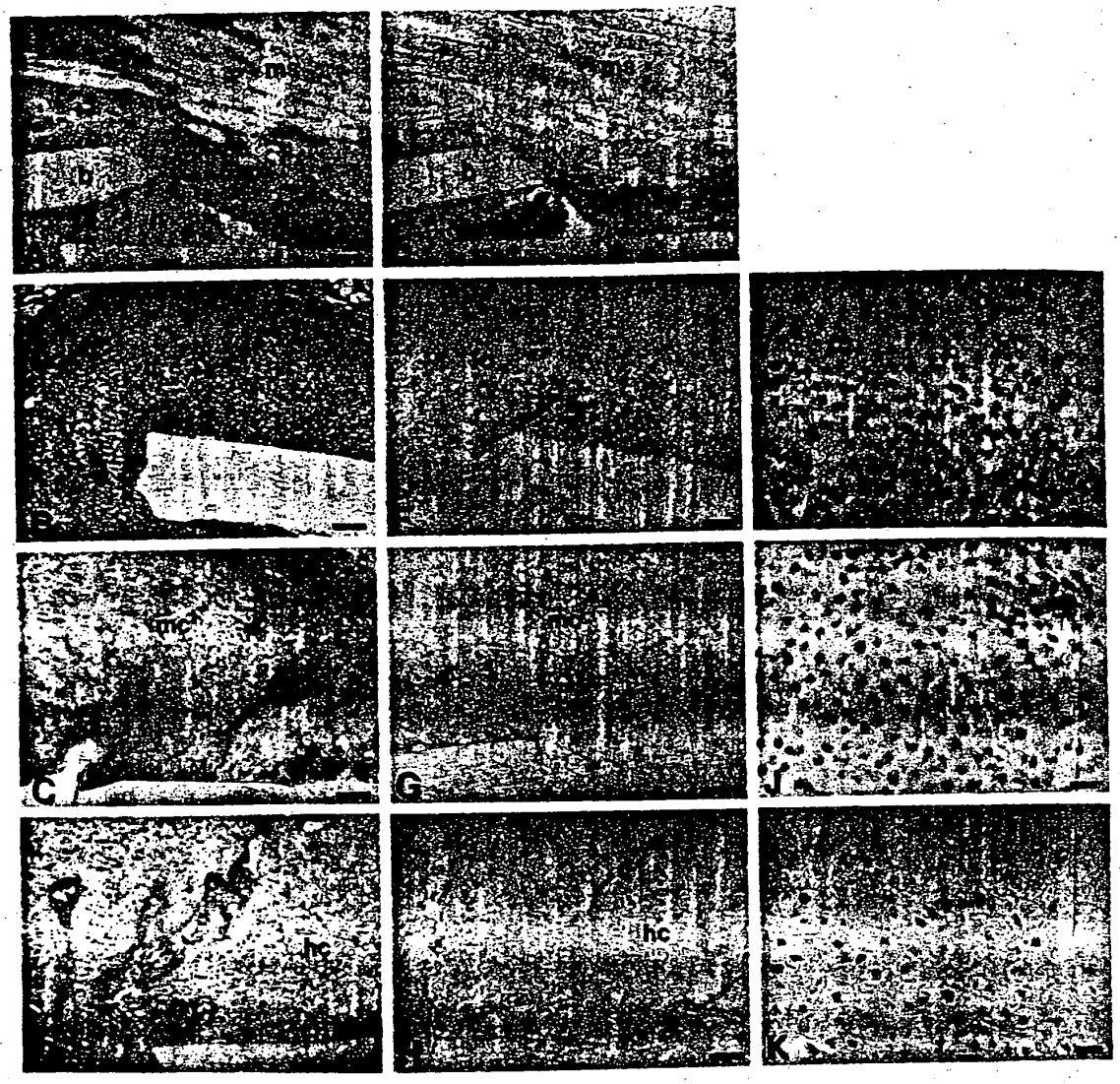

FIG. 5. Photomicrographs showing immunostaining for PCNA (A–D) and Sox9 (E–K). The sections represent days 3 (A, E), 5 (B, F, I), 7 (C, G, J), and 9 (D, H, K) postfracture. The pictures (I–K) show higher magnification of (F–H), respectively. (A) PCNA-positive cells were found around the fracture site. (E) Sox9 could not be detected on day 3. (B) Immature chondrocytes showed intense signals of PCNA. (C) Proliferating cells in mature cartilage showed PCNA signals. (D) As hypertrophy of chondrocytes progresses, PCNA-positive chondrocytes decreased. (F, I) Remarkable signals of Sox9 were found in immature chondrocytes. (G, J) Sox9 was positive in mature chondrocytes. (H, K) With the progress of hypertrophy, Sox9 signals were turned off in hypertrophic chondrocytes. Specific regions and cells were indicated as follows: muscle (ms), cortical bone (b), hematoma (h), immature chondrocytes (ic), mature chondrocytes (mc), hypertrophic chondrocytes (hc). Bar, 100 μm (A–H) and 20 μm (I–K).

In this study, to clarify the specific localization of CD-RAP during cartilage differentiation in the callus, we compared the distribution of CD-RAP with that of type II collagen as a cartilage marker. The distribution of CD-RAP during the fracture repair was basically similar to that of type II collagen. However, the sequential expression of CD-RAP was different from that of type II collagen as follows: on day 5, CD-RAP levels were lower than type II collagen, although both proteins were expressed intensively on day 7 and 9; on day 14, although CD-RAP and type II collagen began to decrease with the progress of hypertrophy, the signal intensity of type II collagen was higher than CD-RAP and more type II collagen was identified in the matrix at the endochondral ossification site; on day 21, even though type II collagen was still detected in cartilage matrix, CD-RAP could not be identified by immunostaining. These findings suggest that CD-RAP has a shorter half-life in the extracellular matrix than type II collagen and may

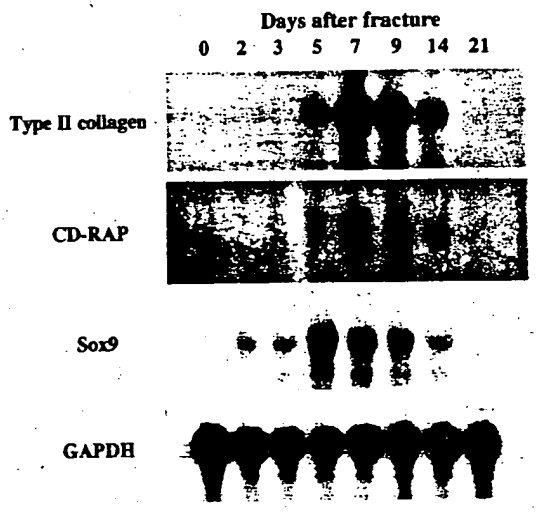

FIG. 6. Autoradiogragh of Northern blot showing the changes in type II collagen. CD-RAP. Sox9. and GAPDH mRNAs during fracture healing. The expression of Sox9 mRNA prior to type II collagen and CD-RAP expression was evident on day 5.

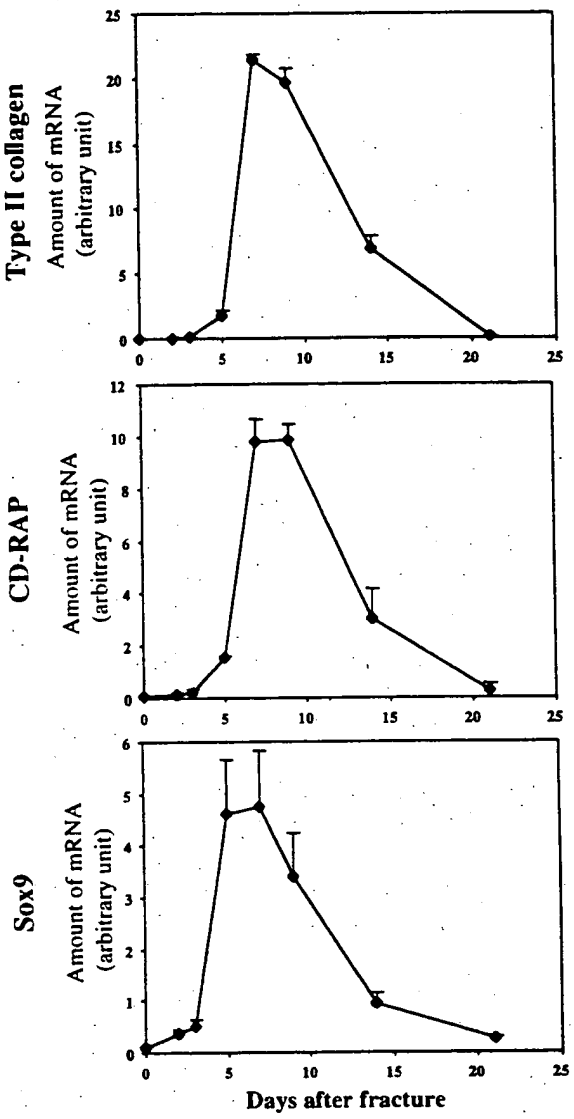

FIG. 7. Graphs showing the changes in the amount of type II collagen. CD-RAP and Sox9 mRNAs during fracture healing. The amount of each mRNA was normalized to GAPDH mRNA. Data are presented as average values and standard error bars are shown.

have a more limited function during fracture healing. Taken together with the results that CD-RAP and type II collagen mRNAs increase and decrease in parallel. CD-RAP may degrade faster than type II collagen or diffuse from the tissue during the progress of hypertrophy.

Another characteristic feature observed in this study was the dramatic change in Sox9 levels in the fracture callus. These changes preceded those identified for CD-RAP and type II collagen. Sox9 is a close relative of the sex-determining region Y protein (SRY) protein. which includes the unique motif of the high mobility group domain.[34] Sox9 is not only critical for determining sex[35,36] but also important for activating chondrogenesis through the enhancement of intron 1 in the type II collagen gene.[23-25] In the present study, we showed that the intense immunostaining for Sox9 on day 5 coincided with the appearance of immature chondrocytes that express low levels of CD-RAP and type II collagen. Northern blot analysis demonstrated advanced expression of Sox9 mRNA on day 5 followed by the expression of CD-RAP and type II collagen mRNAs. The expression of CD-RAP and type II collagen mRNAs decreased with the down-regulation of Sox9 mRNA, accompanied by the progress of hypertrophy and endochondral ossification. Recently, we also demonstrated that Sox9 regulates the promoter activity of the CD-RAP gene by in vitro experiments.[29] Therefore, Sox9 may be controlling both CD-RAP and type II collagen synchronously during fracture healing.

The expression of Sox9 during fracture healing can also be addressed in comparison with the expression of PCNA. PCNA is a well known marker of cell proliferation[37] and several types of cells express PCNA during fracture healing.[38,39] Focusing on the chondrogenic cells, PCNA is expressed in proliferating chondrocytes and decreases in hypertrophic chondrocytes.[38] The expression pattern of Sox9 in chondrocytes in this fracture study correlated well with the expression of PCNA. In the early phase of chondrogenesis on day 5, the cells expressing Sox9 showed PCNA signals. In late phase chondrogenesis (days 9 and 14), with the progress of hypertrophy, the expression of Sox9 was turned off in hypertrophic chondrocytes. This observation coincided with the cessation in the expression of PCNA. Previous studies have also shown that Sox9 expression is increased in proliferating chondrocytes and decreased in hypertrophic chondrocytes. These changes have been demonstrated in embryogenic chondrogenesis and in the growth plate of newborn mice.[26,40,41] Thus, Sox9 may play a role during the proliferation of chondrocytes and in the activation of cartilage-specific genes. These phenomena may represent a decrease in chondrocyte activity with the progress of hypertrophy, which may proceed to the pro-

grammed death of chondrocytes.[39] A previous study suggested the transdifferentiation of chondrocytes to osteoblasts by in situ hybridization, showing the shared expression of types IIA, IIB, and II collagen mRNAs in chondrocytes and osteoblasts.[42] However, in the present study, we identified type II collagen, CD-RAP, and Sox9 expression only in chondrocytes by immunohistochemistry. Further examination is necessary to elucidate the fate of chondrocytes during endochondral ossification.

Observing the prechondrogenic phase during fracture healing, results from our Northern blot analyses revealed more information about the role of Sox9. As shown in Fig. 6, the weak expression of Sox9 mRNA was observed on days 2 and 3 but no type II collagen or CD-RAP mRNA was detected on these days. By immunohistochemistry, no Sox9 protein, CD-RAP, or type II collagen was found on days 2 and 3 at the fracture site. These results suggest that although Sox9 is an important transcription factor for chondrogenesis, low levels of Sox9 mRNA can not produce enough protein to induce the expression of CD-RAP and type II collagen. Therefore, to generate the cartilage phenotype in fracture callus, adequate induction of the Sox9 gene may be necessary to reach the threshold for the expression of cartilage-specific genes. This speculation is supported by the observation that, on day 5, the elevation of Sox9 mRNA resulted in the increase of CD-RAP and type II collagen mRNAs. In addition, it is possible that other transcription factors, which cooperate with Sox9, may be required to induce the chondrocyte phenotype. Our previous data have shown that overexpression of Sox9 increased the endogenous CD-RAP mRNA in chondrocytes in a dose-dependent manner but was unable to induce the endogenous gene in 10T1/2 mesenchymal cells or BALB/c 3T3 fibroblasts.[29] Moreover, Sox9 is not capable of making nonchondrogenic cells express the endogenous type II collagen gene.[24] Recently, it was reported that a new long form of Sox5 (L-Sox5) and Sox6 are coexpressed with Sox9 and cooperatively activate the type II collagen gene.[43] It is feasible that these two transcription factors, L-Sox5 and L-Sox6, may be involved in chondrogenesis at the fracture site. Further studies are necessary to show that genetic regulation by multiple transcription factors during fracture repair is important. A more detailed understanding of transcriptional regulation during fracture healing may lead to the recognition of target genes for the treatment of fracture and the future strategy of genetic manipulation on delayed-union or nonunion fractures.

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Erratum

A correction should be made to the article "Cartilage-Derived Retinoic Acid-Sensitive Protein and Type II Collagen Expression During Fracture Healing are Potential Targets for Sox9 Regulation" by S. Sakano, Y. Shu, and L. J. Sandell published in 15:1891-1901, November 1999. Please note that the photographs for Figure 1 and Figure 4 are reserved. The on-line version of the article reflects this correction. We apologize for this error.